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That I am knowledgeable in the English language and in the language in which the below identified Japanese application was filed, and that I believe that the English translation of the Japanese application No. 2001-161199 filed on May 29, 2001 attached hereto is a true and complete translation of the above-identified Japanese application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date March 22, 2007

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SPECIFICATION

TITLE OF INVENTION:

5 Process for Preparing Micro-array for DNA Analysis

SCOPE OF PATENT CLAIMS:

1. A process for preparing a micro-array for analysis of DNA which comprises the steps of:

10 spotting onto a solid carrier in a predetermined area thereof in which a number of reactive groups are fixed an aqueous solution which contains a thickening agent and probe molecules having a group reactive with the reactive groups of the carrier to produce covalent
15 bonding, the thickening agent being incorporated in an amount to increase viscosity of the solution to a predetermined value and the probe molecules being selected from the group consisting of nucleic acid fragments, oligonucleotides and peptide nucleic acids;

20 incubating the solid carrier having the spotted aqueous solution on the surface, whereby causing a reaction for producing the covalent bondings; and

 washing the surface of the solid carrier with an aqueous medium to remove the thickening agent from the
25 surface of the solid carrier.

2. The process for preparing a micro-array for analysis of DNA defined in claim 1, wherein the aqueous solution to be spotted onto the solid carrier has a viscosity of 2 to 50 mPa·s.
30

3. The process for preparing a micro-array for analysis of DNA defined in claim 1 or 2, wherein the thickening agent is a water-soluble polymer.
35

4. The process for preparing a micro-array for

analysis of DNA defined in any one of claims 1 to 3, wherein the reactive group of the solid carrier is a vinylsulfonyl group and the reactive group of the probe molecule is an amino group.

5

5. The process for preparing a micro-array for analysis of DNA defined in claim 4, wherein the vinylsulfonyl group is provided to the solid carrier by reacting a divinylsulfone derivative with a amino group which has
10 been previously placed on the solid carrier.

6. The process for preparing a micro-array for analysis of DNA defined in any one of claims 1 to 5, wherein the aqueous medium for washing the solid carrier
15 contains a surface active agent.

7. The process for preparing a micro-array for analysis of DNA defined in any one of claims 1 to 6, wherein the aqueous solutions whose viscosity are increased to predetermined viscosity values for each are
20 spotted in two or more predetermined areas on the solid carrier.

8. The process for preparing a micro-array for analysis of DNA defined in claim 7, wherein each of the aqueous solutions has a viscosity essentially identical to each other.

9. A micro-array for analysis of DNA which is
30 prepared by the process defined in any one of claims 1 to 8.

10. A process for preparing a micro-array for analysis of DNA which comprises the steps of:
35 spotting onto a solid carrier in a predetermined area thereof in which a number of groups electrically

chargeable in an aqueous medium are fixed in an aqueous solution which contains a thickening agent and probe molecules having a group electrically chargeable in an aqueous medium to produce electrostatic bonding with the electrically chargeable groups of the carrier, the thickening agent being incorporated in an amount to increase viscosity of the solution to a predetermined value and the probe molecules being selected from the group consisting of nucleic acid fragments, oligonucleotides and peptide nucleic acids;

incubating the solid carrier having the spotted aqueous solutions on the surface, whereby causing a reaction for producing the electrostatic bondings; and

washing the surface of the solid carrier with an aqueous medium to remove the thickening agent from the surface of the solid carrier.

11. The process for preparing a micro-array for analysis of DNA defined in claim 10, wherein the aqueous solution to be spotted onto the solid carrier has a viscosity of 2 to 50 mPa·s.

12. The process for preparing a micro-array for analysis of DNA defined in claim 10 or 11, wherein the thickening agent is a water-soluble polymer.

13. The process for preparing a micro-array for analysis of DNA defined in any one of claims 10 to 12, wherein the electrically chargeable group of the solid carrier is an amino group and the electrically chargeable group of the probe molecule is a phosphoric acid group.

14. The process for preparing a micro-array for analysis of DNA defined in claim 13, wherein the amino group is provided to the solid carrier by treating the solid carrier with an aminosilane coupling agent or a

poly-cation compound.

15 15. The process for preparing a micro-array for
analysis of DNA defined in any one of claims 10 to 14,
5 wherein the aqueous medium for washing the solid carrier
contains a surface active agent.

10 16. The process for preparing a micro-array for
analysis of DNA defined in any one of claims 10 to 15,
wherein the aqueous solutions whose viscosity are in-
creased to predetermined viscosity values for each are
spotted in two or more predetermined areas on the solid
carrier.

15 17. The process for preparing a micro-array for
analysis of DNA defined in claim 16, wherein each of the
aqueous solutions has a viscosity essentially identical
to each other.

20 18. A micro-array for analysis of DNA which is
prepared by the process defined in any one of claims 10
to 17.

DETAILED DESCRIPTION OF INVENTION

25 [Field of art to which the invention belongs]

This invention relates to a process for preparing a
micro-array for analysis of DNA which is favorably em-
ployable for simultaneous analysis of expression, muta-
tion and polymorphism of gene.

30

[Prior art]

Gene structures of various living bodies as well as
the functions of genes in genome scale have been widely
studied. Accordingly, new technology for analyzing the
35 gene functions has been rapidly developed.

A micro-array for DNA analysis (i.e., DNA chip)

which is composed of a solid carrier (i.e., substrate) and a great number of spots of probe molecules such as DNA fragments or oligonucleotide molecules which are aligned and fixed on the solid carrier in separated areas
5 is generally employed not only for base sequencing of a nucleic acid but also analyzing expression, mutation and polymorphism of gene. The analysis of gene information using the micro-array for DNA analysis is employable for the analysis of molecules of living bodies. The analytical data of genetic information are very advantageously
10 employable also for the study of pharmacologically active substances and further for diagnosis and prevention of diseases. Accordingly, it is desired to develop the preparation method of the micro-array for DNA analysis as well as the analytical system of the resulting data.
15

In the procedure for detection of a nucleic acid such as DNA fragment using the micro-array for DNA analysis, a nucleic acid sample (i.e., target nucleic acid) which is equipped with a radioisotope (RI) label or a
20 fluorescent label is brought into contact with the probe molecules in the spots of the micro-array for hybridization. If the target nucleic acid contain a base sequence complementary to the probe molecules, the target nucleic acid is combined with the probe molecules by hybridization. Thus hybridized target nucleic acid is subjected
25 to detection of the signal and the hybridized probe molecule is identified. In more detail, the radioisotope label or fluorescent label on the micro-array is first detected by means of the RI or fluorescence image scanner. The resulting image data is then analyzed. Thus,
30 the analytical procedure using the micro-array for DNA analysis can give a great number of data on the target molecules employing an extremely small amount of the target sample.

35 A micro-array for DNA analysis is prepared generally by synthesizing probe molecules (such as

oligonucleotides) on a solid carrier (which is called "on-chip method") or by fixing onto a solid carrier a number of previously prepared DNA fragments or oligonucleotides. The former on-chip method is performed
5 by synthesizing a number of oligonucleotides by combinatrial synthesis in each of extremely small areas predetermined on the solid carrier (Foder, S.P.A. et al, Science, 251, 767-773(1991)). In the synthetic procedure, photo-lithography and solid synthesis technology
10 are utilized and a protective group is selectively removed by irradiation of light.

In the latter method, the previously prepared probe molecules such as DNA fragments are spotted on a solid carrier and fixed onto the carrier by covalent bonding or
15 ionic bonding. The bonding is generally produced in the manner described below depending on the nature of the probe molecules and solid carriers.

(1) In the case that the probe molecule is a DNA fragment such as cDNA fragment (i.e., complementary DNA
20 fragment which is synthesized using mRNA as template) or a PCR product (i.e., DNA fragment produced from cDNA by multiplication procedure), an aqueous solution of the DNA fragments is spotted on a solid carrier having a coat of a polycation compound (such as poly-lysine or polyethyl-
25 eneimine) by means of a spotting device of a micro-array preparing apparatus so that the DNA fragments can be electrostatically fixed onto the solid carrier utilizing electric charge of each DNA fragment (Schena, M. et al., Science, 270, 467-470 (1995)).

(2) In the case that the probe molecule is a synthesized product (oligonucleotide), a reactive group is previously incorporated into the oligonucleotide. The oligonucleotide having the reactive group is then brought
30 into contact with a solid carrier which has a reactive group on its surface in an aqueous medium using a spot-
35 ting means so that the desired covalent bonding is pro-

duced between the reactive group of the oligonucleotide and the reactive group of the solid carrier (Protein·Nucleic Acid·Enzyme, vol. 43, No. 13, 2004-2011 (1998); Lamture, J.B. et al., Nucl. Acids Res., 22, 2121-2125

5 (1994); Guo, Z. et al., Nucl. Acids Res., 22, 5456-5665(1994)). Examples of the reactive groups to be incorporated into the oligonucleotide include amino, aldehyde, mercapto (-SH), and biotin. On the surface of the solid carrier, a silane coupling agent having amino,
10 aldehyde, epoxy, or the like is coated to place the reactive group on the surface. The fixation of oligonucleotide by covalent bonding is advantageous because it can produce bonding which is highly stable, as compared with the electrostatic bonding of (1).

15 (3) In the case that the probe molecule is PNA, a reactive group is previously incorporated into the probe PNA in the same manner as in the case (2) as above using the oligonucleotide.

The Japanese Patent Application 11-346157 filed by
20 the present applicant describes a process for fixing DNA fragments onto a solid phase substrate via covalent bonding, the solid phase substrate having vinylsulfone groups on its surface which have been provided onto the substrate by bringing disulfone compounds into contact with
25 the solid phase substrate having been treated with a silane coupling agent having an amino group or the like.

The spotting of an aqueous solution of DNA fragments or other probe molecules onto a solid carrier is performed by means of various spotting devices. Generally
30 employed are a pin method in which the aqueous solution is spotted by means of a pin which is brought into contact with the surface of the solid carrier and an ink jet method using an ink jet printing system. In any methods, it is required that plural spots of the aqueous solution
35 are essentially identical to each other in their shapes and sizes so as to assure accuracy of the resulting ana-

lytical data.

United States Patent No. 5,837,196 teaches that probe molecules such as nucleic acids are fixed onto a solid carrier using an aqueous solution of binder polymer (matrix polymer) such as nitrocellulose. The matrix polymer is then kept on the solid carrier after the probe molecules are fixed, so that it is understood that the matrix polymer serves as adhesive to retain the probe molecules on the carrier.

Japanese Patent Publication 2000-22180 filed by the present applicant describes a process for fixing DNA fragments to a solid carrier which comprises spotting an aqueous solution containing DNA fragments and a hydrophilic polymer onto the solid carrier. In this case, the hydrophilic polymer is not removed after the fixation, and the remaining hydrophilic polymer serves to combine the DNA fragments with the solid carrier via electrostatic bonding.

The inventors of the present invention have noted that although the fixation of the probe molecules onto the solid carrier can be stabilized by the use of a hydrophilic polymer, the DNA fragment sample being non-complementary to the probe is apt to adhere to the hydrophilic polymer. The adhesion of the non-complementary sample to the hydrophilic polymer causes an error in the measurement of the DNA fragment sample.

[Problems to be solved by the invention]

It is an object of the present invention to provide a process for preparing a micro-array for DNA analysis, which gives high detection accuracy and low detection error in the analysis of DNA fragment samples using the micro-array for DNA analysis.

[Invention to solve the problems]

The present inventors have discovered that an aque-

ous solution which contains probe molecules such as DNA fragments (which has a reactive group capable of combining with reactive groups previously formed on the solid phase substrate) and a water soluble thickening agent
5 (which is easily washable) in such an amount that the aqueous solution has an increased viscosity forms on a solid carrier a spot of probe molecules which has a desired and uniform size and form with good reproducibility. The inventors have further discovered that when the
10 thickening agent is removed by washing or the like after the probe molecules are fixed onto the solid carrier, fixation of the nucleic acid fragment samples to the solid carrier via other mechanisms than hybridization can be kept, and that, as a result, incorporation of background noises into the measured fluorescence strength is
15 obviated. The present invention has been made on this finding.

It is further observed that the effect caused by the incorporation of a thickening agent into the aqueous
20 solution containing probe molecules is also observed even in the case that the probe molecules are fixed onto the solid carrier via electrostatic bonding.

Accordingly, the present invention resides in a process for preparing a micro-array for analysis of DNA
25 which comprises the steps of:

spotting onto a solid carrier in a predetermined area thereof in which a number of reactive groups are fixed an aqueous solution which contains a thickening agent and probe molecules having a group reactive with
30 the reactive groups of the carrier to produce covalent bonding, the thickening agent being incorporated in an amount to increase viscosity of the solution to a predetermined value and the probe molecules being selected from the group consisting of nucleic acid fragments,
35 oligonucleotides and peptide nucleic acids;

incubating the solid carrier having the spotted

aqueous solution on the surface, whereby causing a reaction for producing the covalent bondings; and

washing the surface of the solid carrier with an aqueous medium to remove the thickening agent from the surface of the solid carrier.

The present invention further resides in a process for preparing a micro-array for analysis of DNA which comprises the steps of:

spotting onto a solid carrier in a predetermined area thereof in which a number of groups electrically chargeable in an aqueous medium are fixed an aqueous solution which contains a thickening agent and probe molecules having a group electrically chargeable in an aqueous medium to produce electrostatic bonding with the electrically chargeable groups of the carrier, the thickening agent being incorporated in an amount to increase viscosity of the solution to a predetermined value and the probe molecules being selected from the group consisting of nucleic acid fragments, oligonucleotides and peptide nucleic acids;

incubating the solid carrier having the spotted aqueous solutions on the surface, whereby causing a reaction for producing the electrostatic bondings; and

washing the surface of the solid carrier with an aqueous medium to remove the thickening agent from the surface of the solid carrier.

In the invention for the process of preparing a micro-array for DNA analysis, the following embodiments are preferred:

(1) The aqueous solution to be spotted onto the solid carrier has a viscosity of 2 to 50 mPa·s.

(2) The thickening agent is a water-soluble polymer.

(3) The reactive group of the solid carrier is a sulfonylvinyl group and the reactive group of the probe molecule is an amino group.

(4) The sulfonylvinyl group is provided to the solid carrier by reacting a divinylsulfone derivative with a amino group which has been previously placed on the solid carrier.

5 (5) The electrically chargeable group of the solid carrier is an amino group and the electrically chargeable group of the probe molecule is a phosphoric acid group.

10 (6) The amino group is provided to the solid carrier by treating the solid carrier with an aminosilane coupling agent or a poly-cation compound.

 (7) The aqueous medium for washing the solid carrier contains a surface active agent.

15 (8) The aqueous solutions whose viscosity are increased to predetermined viscosity values for each are spotted in two or more predetermined areas on the solid carrier.

20 (9) Each of the aqueous solutions has a viscosity essentially identical to each other. The "viscosity essentially identical to each other" means that there is so little difference in the viscosity that the conditions of the spotted aqueous solutions are essentially identical to each other.

25 (10) Coefficient of Variation (CV) of the spots formed by the spotting the aqueous solutions is not higher than 6.5%. The CV value means a coefficient of variation obtained by measuring fluorescence strengths in plural spots formed on the solid carrier. In more detail, the CV can be determined by spotting the aqueous solution(s) containing probe molecules labeled with fluorescent agent Cy (optionally and a water soluble thickening agent) on a solid carrier in plural separated areas using a spotting means (e.g., spotter "CARTESIAN" available from TECHNOLOGIES CORP.), incubating the spotted solid carrier for fixing the probe molecules onto the carrier, and measuring the fluorescence strengths in each
35 of the spotted areas of the solid carrier using a fluo-

rescence scanning apparatus, and calculating an average value of the measured fluorescence strengths to obtain a standard deviation (SD, in terms of %) of each measured value.

5 The micro-array for DNA analysis of the invention is advantageously employed in the detection of complementary DNA fragments by the steps of spotting an aqueous solution which contains a target DNA fragment sample having a fluorescent or radioisotope label on the micro-array for
10 DNA analysis, incubating the sample-spotted micro-array for performing hybridization between the probe molecules and complementary DNA fragments in the sample solution; and detecting strengths of fluorescent or radioisotope label on the micro-array at each spotted area in which
15 the probe molecules are previously fixed.

[Embodiments of the invention]

The whole features of the DNA micro-array techniques including the preparation of DNA micro-array and the detection of nucleic acid fragment samples are described
20 below, referring to the attached drawings.

In Fig. 1, a process of preparing a micro-array for DNA analysis and a process of detecting complementary DNA fragments by hybridization using the micro-array for DNA
25 analysis are schematically illustrated. This figure is incorporated herein by copying from the aforementioned "Protein · Nucleic Acid · Enzyme" vol. 43, No. 13, 2004-2011 (1998).

First, DNA collection (e.g., cDNA, EST, oligonucleotide) 21 is prepared from a data base 11 in which genome, cDNA, EST (i.e., DNA fragment of 200 - 300 bp (base pair) from 3'-terminal) or the like is registered or from a clone collection 12 by PCR multiplication or chemical
30 synthesis. The DNA collection 21 is then dissolved or dispersed in an aqueous medium in combination with a
35 water-soluble thickening agent to give an aqueous solu-

tion having an increased viscosity. Thus viscosity-in-
creased aqueous solution is spotted onto a solid carrier
31a by means of a spotting device. Subsequently, the
solid carrier having the spotted aqueous solution on its
5 surface is washed with water so that the thickening agent
can be removed from the carrier. Thus, a micro-array 31
having probe molecules 31b which is employed for analysis
of DNA fragments is prepared.

Separately, mRNA or genome DNA 51 is taken out from
10 a specimen (e.g., cell or tissue) 41, and a target DNA
fragment (or cDNA) 52 is prepared using the mRNA or ge-
nome DNA 51. The target DNA fragment) 52 is then labeled
with a fluorescent substance 53a to give a labeled DNA
nucleic acid 53. The target nucleic acid 53 may be DNA
15 or RNA. The target nucleic acid 53 is brought into con-
tact with the DNA fragments 31b of the micro-array 31 for
performing hybridization between them. The micro-array
having hybridized double chain molecules 61 on its sur-
face is then scanned with a fluorescence measuring appa-
20 ratus to measure fluorescence strength in each spot.
Thus, image data 71 on the spots of hybridized molecules
are obtained.

From the image data 71, base sequence of the target
nucleic acid obtained, or gene expression profile is
25 prepared. Further, more complicated analysis on mutation
or polymorphism can be done using a data analysis comput-
er program or other data base.

In Fig. 2, the micro-array for DNA analysis after
the hybridization is shown in the form of an enlarged
30 image.

The procedure of fixation of the probe molecules
such as DNA fragments to the solid carrier is described
below in detail, first with reference to the fixation via
covalent bonding.

35 It is preferred that the solid carrier is hydropho-
bic or less hydrophilic carrier. The solid carrier may

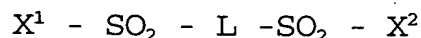
have non-smooth surface having uneven plane. The material of the solid carrier can be ceramics or new ceramics such as glass, cement and ware; polymers such as poly-(ethylene terephthalate), cellulose acetate, polycarbonate of bisphenol A, polystyrene and poly(methyl methacrylate); silicone; porous materials such as active carbon, porous glass, porous ceramics, porous silicone, porous active carbon, woven or knitted fabric, non-woven fabric, filter paper, short fibers, and membrane filter; and metal surfaces such as gold electrode. The pore size of the porous material generally ranges from 2 to 1,000 nm, preferably 2 to 500 nm. In consideration of easiness of surface treatment and easiness of measurement by means of a fluorescence-scanning apparatus, the solid carrier is preferably made of glass or silicone. The thickness of the solid carrier generally ranges from 100 to 2,000 μm .

It is preferred that the solid carrier is coated with a poly-cation (e.g., poly-L-lysine, polyethyleneimine, or polyalkylamine) so as to form a reactive group. The poly-L-lysine coat is preferred. Otherwise, the surface of the solid carrier can be treated with a silane-coupling agent containing an amino group, an aldehyde group, an epoxy group, or a mercapto group. Particularly preferred is a silane-coupling agent having an amino group or a mercapto group. In the case of using the poly-cation, the amino or mercapto group is placed on the solid surface via electrostatic bonding. In contrast, in the case of using the silane-coupling agent, the amino or mercapto group is placed on the carrier surface via covalent bonding is well fixed onto the carrier surface.

A processing of a solid carrier with a silane-coupling agent in combination with coating with a poly-cation is also employable. The electrostatic bonding between the hydrophobic or less hydrophilic solid carrier and the probe molecules such as DNA fragments is acceler-

ated. On the surface of the solid carrier on which the poly-cation treatment is performed, a layer of electric charge-containing hydrophilic polymer or a layer of a cross-linking agent can be placed. By the provision of these layers, the unevenness of the poly-cation treated solid carrier can be reduced. Depending upon the nature of the solid carrier, a hydrophilic polymer can be incorporated into the solid carrier. Thus processed carrier is also preferably employed.

For fixation of the probe molecules onto the solid carrier by covalent bonding, the solid carrier having the reactive group is preferably brought into contact with the below-illustrated disulfone compound, so that the solid carrier have a vinylsulfone group. The solid carrier of this type and the process for fixing DNA fragments are described in the aforementioned Japanese Patent Application 11-22180:



in which each of X^1 and X^2 is a vinyl group and L is a divalent linking group.

The probe molecule can be classified into three groups such as nucleic acid fragments, oligonucleotide, and PNA.

In order to study of expression of gene, cDNA, a part of cDNA, or DNA fragment such as EST is employed. These polynucleotides can be unknown in their sequences and functions. Generally, they are prepared by PCR method using a library or whole genomes of cDNA or genome chosen from the sequences registered in data base as a template to give a PCR product. Probe molecules which have not been multiplied are also employable.

If mutation and polymorphism of gene is studied, various oligonucleotides corresponding to the mutation and polymorphism can be synthesized based on the known

standard sequences. If the base sequences are determined, oligonucleotides (oligo DNA) of 4^n kinds (n is a length of the base) are synthesized and employed. The DNA fragments to be employed are preferably known in their base sequences. If the DNA fragment is an oligo DNA, it preferably is 2 to 50mer, more preferably 10 to 25mer.

The probe molecule can be a peptide nucleic acid (PNA) which is a synthesized nucleic acid whose phosphodiester bonding is replaced with a peptide bonding.

On the terminal of the probe material such as DNA fragment is generally placed a functional group such as amino, imino, hydrazino, carbamoyl, hydrazinocarbamoyl, or carboxyimide, preferably amino. When the DNA fragment is fixed by covalent bonding, a cross-linker is preferably placed between the functional group of the DNA fragment and the phosphate group. The cross-linker preferably is an alkylene group or an N-alkylamino-alkylene group, more preferably is a hexylene group or an N-methylamino-hexylene group, most preferably the hexylene group.

As is described hereinbefore, the fixation of the probe molecule onto the solid carrier can be done not only by the covalent bonding but also by an electrostatic bonding. The fixation of a probe molecule onto a solid carrier by electrostatic bonding is already known. For instance, a cationic group (amino) is placed on the solid carrier by processing a glass surface with an aminosilane coupling agent or coating the glass surface with a polycation (e.g., polylysine, polyethyleneimine), and with thus processed solid carrier is brought into contact a DNA fragment having an anionic group such as phosphoric acid group, or a synthesized oligonucleotide or PNA having an optional anionic group.

Examples of the water-soluble thickening agents which are characteristic features of the invention in-

clude water-soluble synthetic or natural polymers, polyhydric alcohols such as glycerol, and saccharides such as trehalose, sodium alginate, and starch.

5 The water-soluble polymer may be a cationic, anionic, or amphoteric hydrophilic polymer. A nonionic polymer is also employable. Most preferred is a cationic polymer. The polymer preferably has a molecular weight in the range of 10^3 to 10^6 . If the molecular weight is higher than this range, the viscosity exceedingly in-
10 creases so that it give an adverse effect to dispersability of the probe molecule such as DNA fragment and their binding to the solid carrier.

The cationic polymer preferably is a quaternary ammonium polymer. Examples of the quaternary ammonium
15 polymers include poly(1,4-diazoniabicyclo[2.2.2]octane-1,4-diylmethylen-1,4-phenylenemethylene chloride), polyvinylbenzyltrimethylammonium chloride, poly(methylenetri-methylammonium chloride acrylate), and poly(ethylenetri-methylammonium chloride acrylate). Tertiary amine poly-
20 mers such as poly-N-vinylpyrrolidone, polyvinylimidazole, and polyvinylpyrazole are also employed. Most preferred is poly(1,4-diazoniabicyclo[2.2.2]octane-1,4-diylmethylen-1,4-phenylenemethylene chloride).

The anionic polymer preferably has an anionic group
25 such as COO^- , SO_3^- , OSO_3^- , PO_3^- , or PO_2^- . Examples of the anionic polymers include carboxymethylcellulose, cellulose sulfate esters, polyacrylic acid, polymethacrylic acid, polyvinylbenzene sulfonate, and their salts. Most preferred are sodium polyacrylate, sodium polyvinylben-
30 zene sulfonate, and carboxymethylcellulose.

The nonionic polymer preferably is polyacrylamide, polyethylene glycol, polyvinyl alcohol, acetal of polyvi-
nyl alcohol, cellulose, hydroxyethylcellulose, hydroxypropylcellulose, or one of other cellulose derivatives.
35 Preferred are polyacrylamide and polyethylene glycol.

The amphoteric polymer preferably is a protein such

as albumin, gelatin, gelatin derivative, or casein. Most preferred is albumin.

For the fixation of probe molecules onto the solid carrier, the probe molecules and water-soluble thickening agent are dissolved or dispersed in an aqueous medium such as distilled water or SSC (i.e., Standard Salt-Citrate buffer, or brine and citrate buffer) to prepare an aqueous probe molecule solution for spotting. The aqueous probe molecule solution generally has a viscosity in the range of 1 to 100 mPa·s. When the spotting is done by means of a spotter of quill-pin type, the aqueous solution preferably has a viscosity of 2 to 50 mPa·s, more preferably a viscosity of 2 to 20 mPa·s. If the thickening agent is a water-soluble polymer, the polymer is preferably dissolved in an aqueous medium in an amount of 0.1 to 5 wt.%, more preferably in an amount of 0.3 to 3 wt.%. If the thickening agent is a polyhydric alcohol or saccharide, it is preferably dissolved in an aqueous medium in an amount of 5 to 50 wt.%, more preferably in an amount of 10 to 40 wt.%.

Generally, the aqueous solution is once placed on a plastic plate having 96 or 384 wells, and then spotted onto a solid carrier using a spotting means. The spotting means of pin type in which the aqueous solution can be held is generally employed. The pin holding the solution was then brought into contact with the surface of the solid carrier to transfer the solution onto the solid carrier. The pin may be a solid pin which has no groove on its tip or a quill pin which has a groove on its tip. The quill pin is preferably employed. Other known spotting system such as an ink jet system or a capillary system are also utilizable.

The aqueous solution is spotted onto the solid carrier under the condition that each drop of the solution generally has a volume of 100 pL to 1 μ L, preferably 1 to 100 nL. The probe molecules are preferably spotted onto

the solid carrier in an amount of 10^2 to $10^5/\text{cm}^2$. In terms of mol. of the probe molecule, 1 to 10^{-15} moles are placed in each spot. In terms of weight, several ng or less of probe molecules are placed in each spot. The spotting of the aqueous solution is done onto the solid carrier to form a great number of dots (i.e., spots) having almost the same shape and size. It is important to form these dots to have almost the same shape and size, if the hybridization is to be quantitatively analyzed. Several dots are formed separately from each other with a distance of 1.5 mm or less, preferably 100 to 300 μm . One dot preferably has a diameter of 50 to 300 μm .

After the aqueous solution is spotted on the solid carrier, the spotted solution is incubated, namely, for keeping the spotted solution for a certain period of time at room temperature or under warming (at 25-50°C and 70%RH or higher), so as to firmly fix the spotted probe molecules onto the carrier surface. In the course of incubation, UV irradiation or surface treatment using sodium borohydride or a Shiff reagent may be applied to the spotted solution. The UV irradiation under heating is preferably adopted. These treatments are effective to form the desired stable covalent bonding or electrostatic bonding between the solid carrier and the probe molecules.

The solid carrier onto which the probe molecule is fixed is then washed to essentially remove the water-soluble thickening agent from the surface of the solid carrier. The washing of the surface of the solid carrier can be performed with an aqueous sodium dodecylsulfate (SDS) solution or a standard sodium chloride-citric acid buffer solution. The washing solution may be warmed. Thus, the water-soluble thickening agent is essentially removed, and simultaneously the unbounded probe molecule is removed.

The DNA detection micro-array of the invention pre-

pared by the above-mentioned method for fixation of probe molecules has a great number of spots or dots (generally, from several hundreds to tens of thousands) in which the probe molecules are fixed to the solid carrier by covalent bonding or electrostatic bonding. The CV which is relative to variation of conditions of the different spots preferably is less than 6.5%.

The life of the micro-array for DNA analysis prepared by the above-mentioned method for fixation of probe molecules is within several weeks in the case of a micro-array of cDNA analysis having the fixed cDNA. In the case of a micro-array of oligo-DNA analysis having the fixed oligo-DNA, the life is longer. These micro-arrays for the DNA analysis are utilizable for the monitoring of gene expression, determination of base sequence, analysis of mutation, and analysis of polymorphism.

The method for detection of DNA fragment samples using the micro-array for DNA analysis of the invention is described below.

First, a labelled DNA fragment sample is prepared.

The DNA fragment sample (target DNA) generally is a DNA fragment or RNA fragment whose sequence and function are not known.

If the detection is performed for the purpose of study of gene expression using eucaryote, the target DNA fragment can be prepared in the following manner: m-RNA is extracted from a cell or tissue sample of eucaryote, and is converted into cDNA by incorporating a labelled dNTP ("dNTP" is a deoxyribonucleotide in which the bases comprises adenine (A), cytosine (C), guanine (G) or thimine (T)) by reverse transcription reaction. In the case of employing procaryote such as microorganism, the whole RNA is extracted because a selective extraction of mRNA is difficult. In the case of employing genome as the sample, it can be isolated from optional tissue samples other than erythrocyte. Examples of the optional

tissue samples include peripheral blood lymphocyte, skin, hair, and seminal fluid. The amount of mRNA required for one hybridization procedure generally is several μ g or less, though the amount varies on the amount of the sample liquid and the labelling method. There is a method in which mRNA is used as an antisense RNA. The standard dNTP preferably is a labelled dCTP, from the viewpoint of chemical stability. In addition, the DNA fragment on the micro-array for DNA analysis is an oligo DNA, the DNA fragment sample is preferably converted into a low molecular weight product.

The labelled DNA fragment sample can be obtained by performing PCR in the target area in the reaction system containing a labelled primer or a labelled dNTP.

To the target DNA fragment is attached an RI label or a non-RI label (fluorescence, biotin, chemical luminescence) by a known method. The fluorescence label is most preferably employed. Examples of the fluorescence labels include cyanine dyes (e.g., Cy3 and Cy5 belonging to Cy Dye™ series), rhodamine 6G reagent, N-acetoxy-N²-acetylaminofluorene (AAF), and AAIF (iodide derivative of AAF).

The labelled DNA fragment sample is then dissolved or dispersed in an aqueous medium such as SSC to give an aqueous sample solution. The aqueous solution is spotted onto a micro-array for DNA analysis of the invention and incubated for performing hybridization. The spotting is performed on a plastic plate of 96 wells or 384 wells by means of a spotting apparatus. The spotting is generally done in an amount of less than several μ g, preferably in the range of 1 to 100 nL. The hybridization is carried out by keeping the micro-array for DNA analysis having the spotted sample solution thereon at a temperature between room temperature and 70°C, for 6 to 20 hours.

If the case of using the micro-array for DNA analysis, only a small amount of the DNA fragment sample is

required. However, it is necessary to set optimum detection conditions for the hybridization depending on the chain length and kinds of the sample on the micro-array for DNA analysis. If the analysis of gene expression is intended, the hybridization is preferably performed for a long period of time so as to sufficiently detect a gene of low expression. On the other hand, the detection of one-base mutation is preferably performed within a short period of time. Thus, the DNA fragment on the micro-array for DNA analysis is hybridized with a molecule of the labelled DNA fragment sample complementary to the probe, to form a hybrid DNA (double chain).

After the hybridization is complete, the micro-array for DNA analysis is washed with an aqueous buffer solution containing a surface active agent, to remove the unfixed DNA fragment samples. The surface active agent preferably is sodium dodecyl sulfate (SDS). The buffer solution may be a citrate buffer solution, a phosphate buffer solution, a borate buffer solution, Tris buffer solution, or Goods buffer solution. The citrate buffer solution is preferably employed.

The fluorescent signal emitted by the hybrid DNA on the micro-array for DNA analysis is then detected by means of a detector. Examples of the detectors include a fluorescence laser microscope or a fluorescence scanning apparatus connected to a cooled CCD camera and a computer. The detector enables to automatically measure the fluorescence strength on the micro-array for DNA analysis. A co-focusing or non-focusing laser can be utilized in place of the CCD camera. Thus, image data corresponding to the micro-array for DNA analysis are obtained. From the obtained data, the sample DNA fragments complementary to the probe (such as DNA fragment) on the micro-array for DNA analysis can be identified. Based on the obtained results, a gene expression profile can be prepared, or the base sequence of the nucleic acid fragment

sample can be determined. Further, more detailed analysis on the mutation or polymorphism of gene can be performed using a data analyzing software or an outer data base. Otherwise, comparison or quantitative analysis of expression can be using one micro-array for DNA analysis by simultaneously using plural DNA fragment samples having different fluorescence labels.

[Examples 1 to 3] Fixation of DNA fragment (probe molecule) by covalent bonding, and evaluations of spot reproductivity and amount of fixed DNA fragment

(1) Preparation of solid carrier (B) for covalent bonding

A glass plate (25 mm x 75 mm) was dipped in an aqueous solution of 2 wt.% aminopropylethoxysilane (available from Shin-etsu Chemical Industry Co., Ltd.) for 10 minutes and then taken out. The glass plate was subsequently washed with water and dried to 110°C for 10 minutes, so as to prepare a glass plate having a coat of the aminosilane compound. The coated glass plate was dipped in an aqueous borate buffer solution (pH 8.0) containing 3 wt.% of 1,2-bis(vinylsulfonylacetamide)ethane for 2 hours and then taken out from the aqueous solution. The glass plate was washed with water and dried for one hour under pressure, to give a glass plate (B) (for covalent bonding) having on its surface reactive vinylsulfonyl groups.

(2) Fixation of DNA fragment

5'-CTAGTCTGTGAAGTGTCTGATCCTCCCCGGACATGGAGGA-3' (oligonucleotide of 40mers) having a fluorescent label (Fluorolink Cy5-dCTP, available from Amasham Pharmacia Biotec Corp.) at 3'-terminal and 5'-terminal (i.e., Cy5-Oligo DNA(+)) was prepared.

Separately, aqueous solutions containing a water-soluble thickening agent (set forth in Table 1) were prepared. In each aqueous solution was dispersed the Cy5-Oligo DNA(+) in an amount of $1 \times 10^{-6}M$, to prepare an

aqueous Cy5-Oligo DNA(+) solution.

The aqueous solution was spotted on the aforementioned solid carrier (B) having reactive vinylsulfonyl groups by means of a commercially available spotting apparatus (of quill pin type, available from CARTESIAN TECHNOLOGIES CORP.). Fifty spots were placed on the solid carrier (B).

The solid carrier was placed in a moisture chamber having an inner temperature of 25°C and a humidity of 70% for 18 hours. The solid carrier was then taken out from the moisture chamber and dried at room temperature.

(3) Washing of the solid carrier

The above-prepared solid carrier was washed with two portions of a mixture of an aqueous SDS (0.1 wt.%) solution and 2xSSC (Standard Salt-Citrate buffer of brine-citrate buffer) at room temperature, one portion of 0.2xSSC at 50°C, and one portion of distilled water at room temperature, so as to remove the water-soluble thickening agent from the glass plate.

The washed solid carrier was dried to give a solid carrier on which the DNA fragment is formed.

(4) Evaluation of spot reproducibility and fixation of DNA fragment

The solid carrier having the fixed DNA fragment was scanned on its spot areas by means of a fluorometer before or after the washing.

The measured values on the different spots were processed to obtain a standard deviation (%) from an average value (N=50) of a relative fluorescence strength values, that is, CV (coefficient of variation, %), for evaluating the reproducibility of the formed spots. Further, a mean fluorescence strength was obtained in each measurement (i.e., prior to and after the washing) to determine a relative amount (%) of probe molecule remaining in the spots.

[Comparison Examples 1 and 2]

The procedures of Example 1 were repeated except that the spotting of probe molecules was carried out using an aqueous 2xSSC solution or diluted water in place of the aqueous solution containing the thickening agent, to prepare solid carriers having the fixed DNA fragment (probe).

The same evaluations on the spot reproducibility and the fixed DNA fragment amount were performed in the manner described in Example 1.

The results are set forth in Table 1.

Table 1

	Spotting Solution (viscosity: mPa·s)	Fluorescence Strength (CV: %)		
		Prior to washing	After washing	Remain- ing ratio
Ex. 1	1 wt.% CMC (8.5)	43,200 (1.8)	33,100 (2.0)	76.6%
Ex. 2	1 wt.% PAA (12.0)	45,800 (1.6)	36,300 (1.9)	79.3%
Ex. 3	30 wt.% Treh. (3.5)	41,000 (2.2)	30,800 (2.5)	75.1%
Com. 1	2 x SSC (1.1)	38,500 (6.5)	28,200 (7.0)	73.2%
Com. 2	distilled water (0.9)	38,000 (7.3)	28,300 (7.5)	74.5%

Remarks: CMC: carboxymethylcellulose
PAA: polyacrylamide

5 The results set forth in Table 1 indicate that the
spotting solutions of Examples 1 to 3 which contain a
water-soluble thickening agent or a saccharide give spots
(prior to the washing) showing a CV value which is in the
range of $1/2$ to $1/3$, as compared with the corresponding
CV value of the spots given by the use of the convention-
10 al spotting solutions of Comparison Examples 1 and 2.
This means that the process of the invention is effective
for giving well reproducible and satisfactorily round
spots. Further, the spots of the bonded probe molecules
produced by the use of a thickening agent according to
15 the invention well keep their conditions even after the
spots were washed with water. In contrast, the spot on
the conventional micro-array is not round (by observa-
tion) and there are non-stable spots on the micro-array.
The presence of these non-stable spots causes high CV
20 value.

Further, the amount of the DNA fixed onto the micro-
array for DNA analysis prepared by the process of the
present invention apparently are as large as the amount
of that fixed onto the conventional micro-array. This
25 means that the water soluble polymers and saccharides do
not participate in the fixation of the DNA fragment onto
the solid carrier.

[Examples 4 to 6] Fixation of DNA fragment (probe mole-
30 cule) by electrostatic bonding, and evaluations of spot
reproductivity and amount of fixed DNA fragment

The procedures of Example 1 were repeated except for
the following:

the solid carrier (B) for covalent bonding was re-
35 placed with a solid carrier (A) for electrostatic bond-
ing;

the DNA fragment was changed from the Oligo DNA(+) to an Oligo DNA(-) (40 mer) which had the same base sequence but had Cy5 only at 5'-terminal; and

5 the fixation at 25°C, RH 70% in the moisture oven for 18 hours was replaced with fixation by heating to 80°C for one hour and carried out by heating the spotted glass plate to 80°C for one hour and subsequently irradiating the solid carrier with UV rays of 120 mV.

10 The same evaluations on the spot reproducibility and the fixed DNA fragment amount were performed in the manner described in Example 1.

[Comparison Examples 3 and 4]

15 The procedures of Example 1 were repeated except that the spotting of probe molecules was carried out using an aqueous 2xSSC solution or diluted water in place of the aqueous solution containing the water-soluble thickening agent.

20 The same evaluations on the spot reproducibility and the fixed DNA fragment amount were performed in the manner described in Example 1.

The results are set forth in Table 2.

Table 2

5		Spotting Solution (viscosity: mPa·s)	Fluorescence Strength (CV: %) Prior to washing	After washing	Remain- ing ratio
10	Ex. 4	1 wt.% CMC (8.5)	43,200 (1.5)	9,900 (2.9)	20.5%
15	Ex.5	1 wt.% PAA (12.0)	50,500 (1.8)	11,900 (3.1)	23.6%
20	Ex.6	30 wt.% Treh. (3.5)	45,200 (2.5)	2,800 (6.2)	6.2%
25	Com. 3	2 x SSC (1.1)	42,000 (7.4)	2,600 (9.9)	6.2%
	Com. 4	distilled water (0.9)	43,500 (6.8)	2,400 (10.5)	5.5%
30	Remarks:	CMC: carboxymethylcellulose PAA: polyacrylamide			

35 The results set forth in Table 2 indicate that the DNA fragment-fixed solid carrier (Examples 4 to 6) which was prepared by electrostatically fixing the DNA fragment onto the solid carrier using an aqueous DNA fragment solution containing a thickening agent such as a water-soluble polymer or polysaccharide shows a CV value which is in the range of 1/2 to 1/3, as compared with the corresponding CV value of the spots on the conventional DNA fragment-fixed solid carrier (Comparison Examples 3 and 40 4). Accordingly, it is clear that the spotting in the

process of the invention is stably effected to give uniform round spots with high reproducibility. Since the electrostatic bonding to the solid carrier is weak, the removal of the thickening agent by washing with water simultaneously causes local detachment of the DNA fragment. Nevertheless, the amount of the remaining DNA fragments on the washed micro-array is larger than the amount observed in the case (Comparison Examples 3 and 4) of using no thickening agent. Moreover, since the CV of the fluorescence strength of the spots after washing is as low as that of the covalent bonding, it is understood that the spotting is stably performed to give uniform round spots with high reproducibility.

[Examples 7 to 9] Preparation of micro-array for DNA analysis and detection of nucleic acid fragment sample

(1) Fixation of DNA fragments by covalent bonding

5'-TCCTCCATGTCCGGGGAGGATCTGACACTTCAAGGTCTAG-3' (oligonucleotide of 40mers) having an amino group at 5'-terminal (i.e., Oligo DNA(+)) and cDNA of 500 bp having an amino group at 5'-terminal (i.e., cDNA(+)) were both prepared as the DNA fragments.

Separately, aqueous solutions containing a water-soluble thickening agent (set forth in Table 3) were prepared. In each aqueous solution was dispersed the Oligo DNA(+) or cDNA(+) in an amount of $1 \times 10^{-6}M$, to prepare an aqueous solution of Oligo DNA(+) or cDNA(+).

Each of the aqueous solution was spotted on the micro-array for DNA analysis (B) (for covalent bonding which was prepared in Example 1) by means of an apparatus for preparation of micro-array for DNA analysis. Fifty spots were placed on the micro-array for DNA analysis.

Separately, an aqueous solution containing only the water-soluble thickening agent was spotted on the solid carrier for preparing a negative control.

After spotting, the solid carrier was placed in a

moisture chamber having an inner temperature of 25°C and a humidity of 70% for 18 hours. Subsequently, the solid carrier was immersed in a 0.5 M aqueous glycine solution (pH 8.5), whereby fixing the DNA fragment onto the solid carrier.

(3) Washing of the solid carrier

These solid carrier were dipped in a boiling water for 3 minutes, so as to remove the water-soluble thickening agent. Immediately, the solid carrier was dipped in an ice-chilled ethanol and dried at room temperature to give a micro-array for DNA analysis B' (in which the DNA fragments were fixed onto the micro-array for DNA analysis).

(4) Hybridization

A 500 bp DNA fragment sample having a fluorescence label Cy5 at its 5'-terminal which has a base sequence complementary to the Oligo DNA(+) and which is also complementary to the cDNA(+) was prepared.

Thus prepared DNA fragment sample was dispersed in 20 µL of an aqueous solution (mixture of 4xSSC and 10 wt.% of aqueous SDS solution) for hybridization, and heated to 90°C for 3 minutes. The heated solution was then cooled and spotted on the micro-array for DNA analysis prepared in (2) as above using a quill pin spotting device. The surface of the micro-array was covered with a cover glass (for microscopy) and the micro-array was placed in a moisture chamber and incubated at 60°C for 20 hours. The incubated micro-array was then washed with two portions of a mixture of an aqueous SDS (0.1 wt.%) solution and 2xSSC at room temperature, one portion of 0.2xSSC at 50°C, and one portion of distilled water at room temperature.

The washed micro-array for DNA analysis was centrifuged at 600 r.p.m. for 20 seconds for removing water and dried at room temperature.

(5) Detection of nucleic acid fragment sample and evaluation of spot reproducibility

The micro-array for DNA analysis was scanned on its sport area by a fluorometer for detecting the labelled DNA fragment sample. The measured values were processed to obtain a mean relative value of the spots. Further, a coefficient of variation (CV, %, N=50) of the spots was obtained, for evaluating the reproducibility of the formed spots.

[Comparison Examples 5 and 6]

The procedures of Example 7 were repeated except that the spotting of probe molecules was carried out using an aqueous 2xSSC solution or diluted water in place of the aqueous solution containing the water-soluble thickening agent, to prepare micro-array for DNA analysis (in which the DNA fragment sample was fixed onto the solid carrier).

Subsequently, the detection of nucleic acid fragment sample and evaluation of the spot reproducibility were performed.

The results are set forth in Table 3.

Table 3

5	Spotting Solution		Fluorescence Strength		
	(viscosity: mPa·s)		Fixing of Oligo DNA(+)	Fixing of cDNA(+)	Negative Control
10	Ex. 7	1 wt.% CMC (8.5)	18,600 (2.5)	24,600 (2.4)	0
15	Ex. 8	1 wt.% PAA (12.0)	20,200 (2.7)	26,600 (2.1)	0
20	Ex. 9	30 wt.% Treh. (3.5)	16,700 (3.5)	23,100 (3.2)	0
25	Com. 5	2 x SSC (1.1)	15,500 (8.2)	22,200 (9.2)	0
30	Com. 6	distilled water (0.9)	15,600 (7.9)	20,300 (8.6)	0
Remarks: CMC: carboxymethylcellulose PAA: polyacrylamide					

35 The results set forth in Table 3 indicate that the micro-array for DNA analysis prepared by the use of spotting solutions which contain a water-soluble polymer or saccharide (Examples 7 to 9) give spots giving a high fluorescence strength and showing a CV value which is in the range of 1/2 to 1/3, as compared with the corresponding CV value of the spots given by the use of the conventional spotting solutions of Comparison Examples 5 and 6, in the fixation of not only an oligo DNA but also its cDNA. This means that the process of the invention is

effective for giving stable, well reproducible and uniform round spots. As a result, it is apparent that the micro-array for DNA analysis prepared by the process of the invention is employed for detecting the DNA fragment sample with a high accuracy.

5 In addition, there was only a small difference of fluorescence strength among the cases using different water-soluble thickening agents, and it was confirmed that the fluorescence strength of the negative control
10 was 0. As a result, it is understood that each of the thickening agents such as water-soluble polymers and polysaccharides is removed by the washing, almost does not pertain in the fixation of the DNA fragment onto the solid carrier, does not disturb the hybridization, and
15 does not give adverse effects to the detection of the sample.

[Comparison Examples 7 to 9]

The procedures of Example 7 was repeated to prepare
20 a micro-array for DNA analysis in which the DNA fragment was fixed onto the solid carrier) except that the step (2) for washing the solid carrier was not done.

The prepared micro-array for DNA analysis was then subjected to detection of the DNA fragment sample and the
25 evaluation on the spot reproducibility.

The results together with the results obtained in Examples 7 to 9 are set forth in Table 4.

Table 4

5		Spotting Solution (viscosity: mPa·s)	Washing of Spots with Water	Fluorescence Strength (CV: %) Oligo DNA(+)	Negative Control
10	Ex. 7	1 wt.% CMC (8.5)	Done	18,600 (2.5)	0
15	Ex. 8	1 wt.% PAA (12.0)	Done	20,200 (2.7)	0
20	Ex. 9	30 wt.% Treh. (3.5)	Done	16,700 (3.5)	0
25	Com. 7	1 wt.% CMC (8.5)	None	10,300 (6.6)	300
30	Com. 8	1 wt.% PAA (12.0)	None	13,500 (5.3)	100
	Com. 9	30 wt.% Treh. (3.5)	None	15,800 (3.8)	0
35	Remarks:	CMC: carboxymethylcellulose PAA: polyacrylamide			

40 The results set forth in Table 4 clearly indicate that each of the micro-array for DNA analysis (Examples 7 to 9) prepared by washing the water-soluble thickening agent according to the process of the invention shows higher fluorescence strength and better spot reproducibility than the micro-array for DNA analysis

(Comparison Examples 7-9) which were not subjected to the washing for removal of the thickening agent. Particularly, if the thickening agent is a water-soluble polymer, the fluorescence strength is prominently high, the spot reproducibility is prominently good, and the sample is detected with increased accuracy.

In contrast, the fluorescence was observed on the spots even in the negative control if the washing was not done (Comparison Examples 7 and 8), whereby disturbing the detection of the sample with a high accuracy. It is considered that the labelled DNA sample was non-specifically adsorbed by the water-soluble polymer so that the fluorescence strength of the negative control was not 0.

[Effects of invention]

According to the process of fixing a probe molecule employed in the process of the invention for preparing a micro-array for DNA analysis, a spot of an aqueous probe solution formed on a solid carrier is stabilized by incorporating a removable water-soluble thickening agent into the aqueous solution. Further, the incorporation of the thickening agent prominently improves the spot reproducibility by adjusting the shapes and sizes of the spots. Since the thickening agent is substantially removed after the fixation of the probe molecule, it does not disturb the contact between the probe and a DNA fragment sample in the hybridization procedure and the efficiency of hybridization does not lower. Further, the background fluorescence strength extremely lowers. Therefore, the accuracy in the detection of a DNA fragment sample is prominently improved.

BRIEF DESCRIPTIONS OF DRAWINGS

Figure 1 is a schematic view of total DNA micro-array technology.

Fig. 2 is an enlarged schematic view of the DNA

micro-array after hybridization.

	11	database
	12	clone collection
	21	DNA collection
5	31	DNA micro-array
	31a	solid carrier
	31b	DNA fragment
	41	test sample
	51	mRNA or genome DNA
10	52	cDNA or target DNA
	53	labelled target nucleic acid
	53a	labelling material
	61	DNA micro-array on which hybrid DNA is formed
	71	image data
15		

FIG. 1

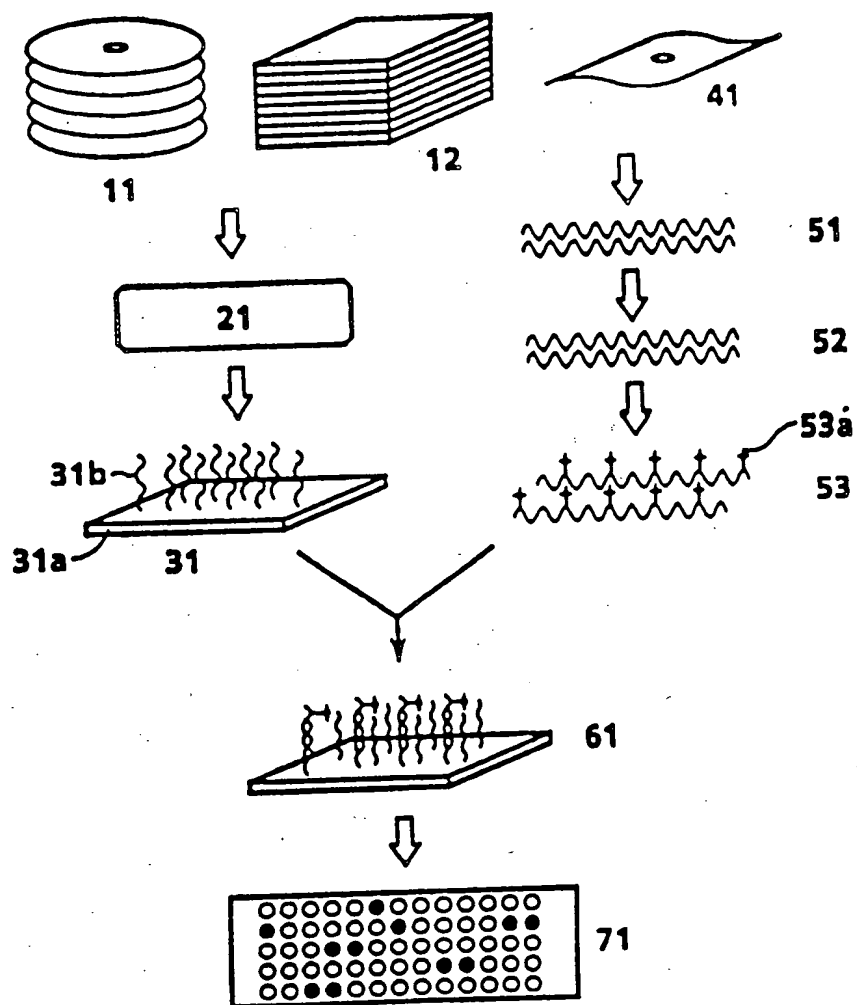
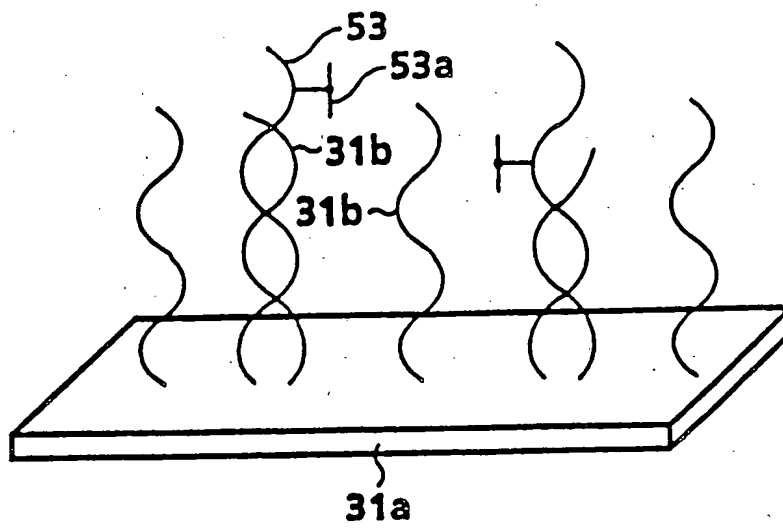


FIG. 2



ABSTRACT OF THE DISCLOSURE

[Object] To provide a process for preparing a DNA micro-array that enables to detect a DNA fragment sample with a high accuracy and a low error.

[Invention] A process for a micro-array for DNA analysis which comprises the steps of spotting an aqueous solution onto a solid carrier in its predetermined area in which plural reactive groups are fixed, the aqueous solution containing a thickening agent and probe molecules (e.g., DNA fragments) having a group reactive with the reactive group of the solid carrier to produce covalent bonding; incubating the aqueous solution-spotted solid carrier to produce the covalent bondings; and washing the solid carrier with an aqueous medium to remove the thickening agent from the solid carrier. The process for preparing a micro-array for DNA analysis utilizing a thickening agent can be favorably applied to the preparation of a micro-array in which the probe molecules are fixed onto the solid carrier via electrostatic bonding.

[Drawing to be selected] None